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Systematic Position of *Hymenophyllum rolandi-principis* Rosenst. or a Monotypic Genus *Rosenstockia* Copel. (Hymenophyllaceae) Endemic to New Caledonia

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Systematic position of *Hymenophyllum rolandi-principis*, or a monotypic genus *Rosenstockia*, has been traced by a combination of comparative morphology, cytogenetics, and molecular systematics. It is concluded that this filmy fern has a close relationship to *H. dimidiatum* in spite of its peculiar phenetic characters. Along with that, taxonomical characters of *Hymenophyllum* *s.l.* are preliminarily re-evaluated.

Key words: *accD*, cytogenetics, *Hymenophyllum rolandi-principis*, molecular systematics, morphological characters, New Caledonia, *rbcL*, *Rosenstockia*

The genus *Rosenstockia* was described by Copeland (1947) as his 34th genus of the Hymenophyllaceae based solely on *Hymenophyllum rolandi-principis* Rosenst., a species endemic to New Caledonia. This sole species was first described by Rosenstock (1910) based on *Franc 1422* collected at 700 m altitude of Mt. Tao, New Caledonia, in January, 1910 and was deposited in Prince Roland Bonaparte's Herbarium from which the epithet was derived. Rosenstock (1910) gave a full description of vegetative characters of the materials which had no sori on them. Because of the absence of sori, however, he was not sure whether he should place this 'einen isolierten Type' in *Hymenophyllum* or in *Trichomanes*. In consequence, he placed it in the former. The soral character was added by Compton (1922), based on his own collection from Mt. Panié in northern New Caledonia: the sorus has a bivalvate involucre, and Rosenstock's position (1910) was thus certified.

Later this species was referred to by Copeland (1937), who had never seen its fertile materials, in his monograph on *Hymenophyllum* (Copeland 1937). When establishing his splitting system of Hymenophyllaceae (Copeland 1938), he could not relate this particular species to any of his 33 other genera. He therefore described a new monotypic genus based solely on this species in his comprehensive *Genera Filicum* (Copeland 1947); as to the soral character, he depended on Compton's description (1922). Copeland (1947) made no mention of the reason why he proposed that *Hymenophyllum rolandi-principis* should form a monotypic genus. It is reasonable to think that he considered the features on pinnae distinct from the other species. As stated below, however, any morphological character of the species under consideration is not so unique as to warrant to form a distinct monotypic genus.

Morton (1968) followed Copeland (1947) in treating *Rosenstockia* as a monotypic genus even in his 6 genera system of Hymenophyllaceae. Pichi Sermolli (1977), dividing Hymenophyllaceae into 42 genera, also recognized *Rosenstockia* as a monotypic genus. In the tree of his phyletic scheme, *Rosenstockia* is placed in a position more basal than *Mecodium, Meringium, Sphaerocionium* and the other genera belonging to *Hymenophyllum s.l.* Tryon & Tryon (1981) did not recognize the peculiarity of this species and placed it under *Hymenophyllum*. Iwatsuki (1984, 1990) placed *Rosenstockia* as a monotypic subgenus of *Hymenophyllum*, and compared it with subgen. *Craspedophyllum* sect. *Corrugatae*. These systematic discussions were based on the same collections distributed among various herbaria, and no new observation has been made on the taxonomic characters of this unique species ever since.

Hymenophyllum rolandi-principis is recorded only from northern part of New Caledonia: Mt. Tao and Mt. Panié. This means that its habitat is limited to Mt. Panié mountain range since Mt. Tao said to be a name given to one of the peaks in the mountain range or a substitute for Mt. Panié itself. Mt. Panié is the highest mountain in New Caledonia with an altitude of 1629 m. An annual precipitation of 8000 mm is recorded in this mountain range (Schmidt 2000). The other parts of this island have less rainfalls and are covered with tropical red soils. The island itself has a number of endemic species, and the Mt. Panié mountain range whose ground consists of micaschist also has a unique vegetation with quite a few endemic species including *Agathis montana* and *Araucaria schmidii* (Jaffré 1995, Setoguchi et al. 1998).

We collected sufficient materials of this species on Mt. Panié between 24 and 26 December, 2000, and made a careful analysis to trace their systematic position. The type locality, Mt. Tao, is supposed to be in the same mountain range as Mt. Panié. *Hymenophyllum rolandi-principis* was recorded between 700 m and 1300 m altitude on the herbarium specimens and in the literature, while the type material was collected at 700 m, the lowest altitude. In recent years, molecular analysis using DNA sequence data has been employed in many phylogenetic studies, and has been proved useful (cf. Hasebe *et al.* 1995). However, comparison of morphological characters is still a powerful tool for phylogenetic and systematic studies because of their rich information (cf. Sano *et al.* 2000). In these study fields, it is desirable to use all the available data because hypotheses should be supported by strong correlation between the molecular and morphological characters. In this paper, we provide the result of close observation of morphological characters as well as cytological and molecular analyses of this little known fern species.

Materials and Methods

The ecological and morphological characters were observed in the fields and collected materials were examined under the microscope in the laboratory. The collections of this species in various herbaria had been observed by one of the authors, Iwatsuki, in his study of Hymenophyllaceae over a long period of time.

For counting chromosome number, fronds with sorus in different stages of maturation were fixed in acetic acid - alcohol (1:3) on the day when they were collected in the field. The fixed materials were kept at -20°C after they were brought back to the laboratory. The observation of chromosomes was made by usual squash method in which chromosomes were stained with aceto-carmine.

Eleven species of Hymenophyllaceae were compared with *Hymenophyllum rolandi-principis* using molecular and morphological approaches. We examined ten species of *Hymenophyllum s.l.*, from as wide a range as possible, and one species which belongs to *Trichomanes s.l.* (*Crepidomanes latealatum*) as an out-group material. The materials are from East and Southeast Asia as well as from New Caledonia (Table 1). All the voucher specimens are preserved in TI.

For molecular analysis, fresh materials were dried with silica gel in plastic bags in the field. They were stored at -80°C until used in the analysis. The extraction of total DNA was performed by modified CTAB Method (Hasebe & Iwatsuki 1990), a small quantity of leaf tissue was ground in liquid nitrogen, and put in 800 μl of CTAB buffer. Finally the obtained pellet was dissolved in 50 μl of TE buffer. After quantification, the total DNA was diluted to approximately 10 ng/μl with sterile water. We analyzed DNA sequences of two contiguous areas in chloroplast DNA. One of them is *rbcL* (ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit) gene, which is usually used in fern phylogeny. Another is the region from *rbcL* gene to *accD* (acetyl-CoA carboxylase beta subunit) gene, including the tail part of *rbcL* gene, *rbcL-accD* intergene spacer and part of *accD* gene (Fig. 1; hereafter called '*accD* +IGS'). PCR amplification of *rbcL* gene performed with primers aF and cR (Hasebe *et al.* 1994), 35 cycles: 94°C (45 sec), 52°C (45 sec), 72°C (75 sec). PCR amplification of *rbcL to accD* region performed with primers *rbcL*1364 NF (5'-GATCTTGCTCGCGAGGGTAAT-3') and *accD*816R (5'-CCATGATCGAATAAAGATTCAGC-3'), 35 cycle: 94°C (45 sec), 52-55°C (45 sec), 72°C (75 sec). The PCR products were electrophoresed in 1.0% Seaplaque GTG agarose (Takara), and were cut out into gel blocks with object bands. The DNA was recovered by phenol extraction (twice), chloroform: isoamyl alcohol (24:1) extraction, and ethanol precipitation. After cycle sequencing reaction with BigDye Terminator (Applied Biosystems), each sample was sequenced using ABI 310 genetic analyzer (Applied Biosystems). Obtained sequences were aligned by ClustalX (1.81) with some manual revision, consequently 1206 bp of *rbcL* and 1367-1390 bp of *accD* +IGS region sequences were used for the following analysis. The sequence data were analyzed by maximum parsimony (MP) method, using PAUP*4.0b8. Gaps were handled as missing data, and 1000 times of bootstrap tests were performed.

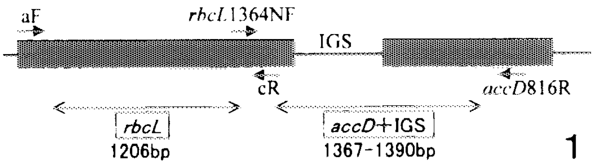


FIG. 1. Sequenced regions (two-headed arrows) and amplifying primers (short arrows) in this study.

Results

Ecology

On Mt. Panié, we observed the altitudinal distribution, and recorded that this species grows between 850 m and 1450 m altitude along the summit trail. *Hymenophyllum rolandi-principis* is basically epiphytic on tree trunks with rare occurrence of epipetric individuals, and is always embedded in a mass of bryophytes in the mossy zone, often with drops of dew on the surface of the leaves (Figs. 2-3). The species grows on a

TABLE 1. List of examined samples with their taxonomical treatments by some different systems

Species	Copeland (1938, 1947)	Morton (1968)	Iwatsuki (1982, 1984)	Locality	Voucher	GenBank Accession No. *rbcL* / *accD*
Hymenophyllum rolandi-principis Rosenst.	*Rosenstockia*	*Rosenstockia*	*Hymenophyllum* subgen. *Rosenstockia*	Mt. Panie, New Caledonia Alt. c. 1300m	*Ebihara 001225-11*	AB064286/AB064298
H. barbatum (Bosch) Baker	*Hymenophyllum*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Hymenophyllum*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Hymenophyllum*	Hachijyo Isl., Tokyo, Japan Alt. c. 750m	*Ebihara 000319-01*	AB064287/AB064299
H. deplanchei Mett.	*Hemicyatheon*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Ptycophyllum*?	*Hymenophyllum* subgen. *Chilodium* sect. *Chilodium*	Mt. Panié, New Caledonia Alt. c. 600m	*Ebihara 001224-03*	AB064288/AB064300
H. dimidiatum Mett.	*Meringium*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Ptycophyllum*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Hymenophyllum*	Mt. Panié, New Caledonia Alt. c. 1400m	*Ebihara 001225-08*	AB064289/AB064301
H. subdimidiatum Rosenst.	*Meringium*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Ptycophyllum*	*Hymenophyllum* subgen. *Chilodium* sect. *Chilodium*	Mt. Panié, New Caledonia Alt. c. 650m	*Ebihara 001226-01*	AB064290/AB064302
H. acanthoides (Bosch) Rosenst.	*Meringium*	*Hymenophyllum* subgen. *Hymenophyllum* sect. *Ptycophyllum*	*Hymenophyllum* subgen. *Chilodium* sect. *Chilodium*	Mt. Kinabalu, Sabah, Malaysia Alt. c. 1500m	*Ebihara Kinabalu030*	AB064291/AB064303
H. fuscum (Blume) Bosch	*Amphipterum*	*Hymenophyllum* subgen. *Mecodium* sect. *Mecodium*	*Hymenophyllum* subgen. *Chilodium* sect. *Amphipterum*	Mt. Gede, Java, Indonesia	*M.Ito 2000 0210-16*	AB064292/AB064304
H. oligosorum Makino	*Mecodium*	*Hymenophyllum* subgen. *Mecodium* sect. *Mecodium*	*Hymenophyllum* subgen. *Chilodium* sect. *Pseudomecodium*	Mt. Haruna, Gunma, Japan Alt. c. 900m	*Ebihara 001105-01*	AB064293/AB064305
H. wrightii Bosch.	*Mecodium*	*Hymenophyllum* subgen. *Mecodium* sect. *Mecodium*	*Hymenophyllum* subgen. *Craspedophyllum* sect. *Integra*	Kirigamine, Nagano, Japan Alt. c. 1500m	*Ebihara 000901-01*	AB064294/AB064306
H. polyanthos (Sw.) Sw.	*Mecodium*	*Hymenophyllum* subgen. *Mecodium* sect. *Mecodium*	*Hymenophyllum* subgen. *Craspedophyllum* sect. *Integra*	Mt. Nachi, Wakayama, Japan Alt. c. 200m	*Ebihara 991122-01*	AB064295/AB064307
Sphaerocionium pilosissimum (C. Chr.) Copel.	*Sphaerocionium*	*Hymenophyllum* subgen. *Sphaerocionium* sect. *Sphaerocionium*	*Sphaerocionium* subgen. *Sphaerocionium*	Mt. Kinabalu, Sabah, Malaysia Alt. c. 1500m	*Ebihara Kinabalu031*	AB064296/AB064308
Crepidomanes latealatum (Bosch) Copel.	*Crepidomanes*	*Trichomanes* subgen. *Trichomanes* sect. *Crepidomanes*	*Crepidomanes* subgen. *Crepidomanes* sect. *Taschnaria*	Uchiura, Chiba, Japan Alt. c. 200m	*Ebihara 991205-02*	AB064297/AB064309

variety of trees, including some gymnosperms, usually up to 1 m above the ground. Fertile materials are rare in the herbaria though they are not so in the fields.

Morphological characters

Peculiar morphology of pinnae can be compared with wings of insects (Figs. 3-5), and such an appearance may be introduced by a collection of veinlets apex in the lobes of upper margin of dimidiate pinnae and by thick and dark veinlets.

The following is a brief description of the taxonomic characters of *Hymenophyllum rolandi-principis*, which is suggestive of the systematic position of this species:

Roots - As is common in the Hymenophyllaceae, roots of this species are borne on rhizome on all sides of protostele and then outside the rhizome direct downwards. Roots are seemingly dark-brown and hard on the surface, with not very densely covered rhizoids which are pale brown to brown. Nothing is particularly unique in these features of the construction of roots.

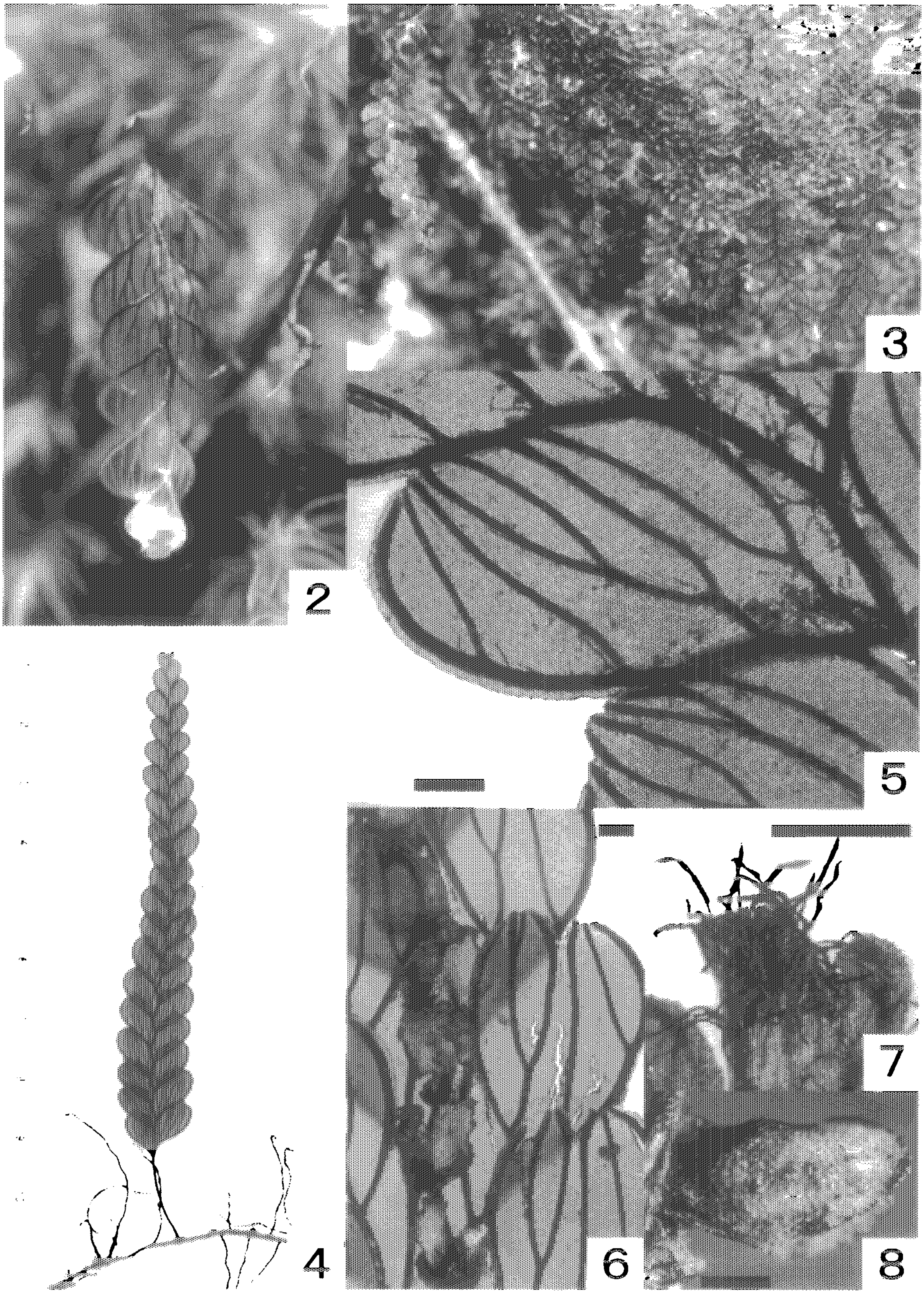
Rhizome - Rhizome is filiform, long-creeping, dully and irregularly branching, and bearing roots, not very abundant, about 0.2 mm in diameter, and often very long. The surface is brown and more or less densely covered with pale brown hairs. Inner construction is simple, which is typical of this family with so-called Hymenophyllaceaen protostele. Hairs on the rhizome are multicellular, usually with 2-4 cells, straight but not very setose, up to 1 mm long, but often destroyed, very dense on the apex but caducous according to its development.

Fronds - Fronds are remotely placed on the rhizome, usually 2-4 (-5) cm from each other, simply-pinnate in construction, with 6-12 (-20) pairs of pinnae, 3-6 (-12) cm long, up to 1.5 cm wide in the widest lower portion. The outline of fronds is narrowly lanceolate, moderately acute at apex, and cuneate at base. Stipes are terete, 1.5-3.5 (-5) cm long, dark-brown, with hairs dense at base and sparse upwards. Hairs are similar to those on the rhizome.

Rachis is similar to the upper portion of stipes, straight, thinner, more densely covered with hairs on the under side.

Pinnae are round-orbicular or roundly subquadriangular in outline, alternately and obliquely placed on rachis, round at apex, cuneate and sessile (or adnate in smaller ones) at base, usually about 1 cm long, 0.5 cm broad, but very variable in size. Pinnae are in general dark green in laminar portion, with thickened and dark brown veins, but whitish-green when unfolding (Fig. 6). The basiscopic half of pinnae is dimidiate, or costae bear only acroscopic veins. Veins are usually in groups of 3-6, with the posterior ones biforked or pinnately tri- or up to 8-forked, and the distal ones simple. Each vein group forms a shallow lobe of pinna, and the apex of veinlets come together at the apex of lobe but not actually unite with each other. The basal margin of pinnae is entire with costa and a few extra-costal rows of cells, and the acroscopic margin is with 3-6 lobes having incised apex according to the apex of veinlets. On the costae and veinlets underneath are sparsely brownish, multicellular hairs up to 1 mm long. Pinna margin is surrounded by veinlets or costae, except for the apex of lobes where the apices of veinlets meet, and there are 3-5 rows of cells outside these submarginal costae and veinlets. Submarginal cells are often irregularly arranged forming denticulation at margin, though these cells are easy to decay in matured fronds.

Trichomes - Hairs are on every axis of matured fronds, or on rhizome, stipes, rachis, costae and veins as described above, and are thick, brownish to pale-brownish, multicellular, and up to 1 mm long but often caducous



FIGS. 2-8. *Hymenophyllum* (*Rosenstockia*) *rolandi-principis*. 2. A young frond in the fields. (Mt. Panié, alt. c.1300m), 3. Ecology; epiphytes on a tree trunk. (Mt. Panié, alt. c.1300m), 4. Sterile frond., 5. A pinna with dark, thickened veins., 6. A fertile frond., 7. Brown, multicellular, hairs on an unfolding frond, 8. A sorus (at a relatively young stage) with bivalvate lips. Bar=1mm.

and/or decaying in older fronds (Fig.7). They are rather dense in younger portions but sometimes glabrescent in older fronds.

Sori - Sori are terminal in axial segment, with a single sorus on each pinna. Involucres are ovate, bivalvate, cleft to the middle (Fig. 8). Lips are minutely denticulate, though old fronds have lips of sori with remarkable denticulation. Receptacles are included at the young stage, though extruding from involucres at the older stages. In older sori, however, the involucres are often decayed and seemingly included.

Cells - Internal cell walls are relatively thick and straight, but not particularly coarsely pitted.

Comparison of morphological characters

Soral character, nature of hair and thickness of cell walls were compared among eleven species of *Hymenophyllum s.l.* including *Hymenophyllum rolandi-principis* (Table 2, Figs. 9-17). Some of the observations on cell wall structure were referred to Copeland (1937).

Cytological observation

In meiosis, chromosomes were counted as $n=21$ (Figs. 18-19). This number suggests that *Hymenophyllum rolandi-principis* should be a diploid species.

Molecular analysis

The resulting trees are shown in Figs. 20 and 21. As for *rbcL* tree, a single most parsimonious tree with 189 steps, having a consistency index (CI) of 0.89 and a retention index (RI) of 0.64 was obtained. As for *accD*+IGS data, two trees of 341 steps with CI=0.89 and RI=0.65 are obtained. These trees do not essentially contradict each other in the topology. Thus, we analyzed combined data of *rbcL* and *accD*+IGS, and obtained a single most parsimonious tree (Fig. 22) with 530 steps (CI=0.88 and RI=0.65). In the tree, most of the clades are supported with high bootstrap value (≧89), with one exception of *H. subdimidiatum* + *H. fuscum*.

Discussion

Dimidiate pinnae is not very rare in Hymenophyllaceae, as seen in *Hymenophyllum dimidiatum* and *H. subdimidiatum* which are included in this study, or in Morton's (1968) subgenus *Hymenophyllum* sect. *Eupectinum* in Neotropic. It can be reminded that *Hymenasplenium* is a derivative of *Asplenium* with dimidiate pinnae in wet environment, having a few (at least two) cell layers of laminae.

As to Hymenophyllaceae, usefulness of *rbcL* gene for phylogenetic analysis was discussed by Dubuisson (1997), especially about a large sister group, *Trichomanes s.l.* The data show many genetic variations not only at an intergeneric level, but also at an infrageneric level. On the contrary, our data (Ebihara, in preparation) and Pryer *et al.* (2001) show that *Hymenophyllum s.l.* has less variation in the construction of phylogenetic tree in *rbcL* gene. Thus, we had selected additional chloroplast sequences of *accD*+IGS region (total approximately 1400 bp). Inamura *et al.* (2000) showed this region has much variation even within a species of Japanese *Polygonum cuspidatum*. As for Hymenophyllaceae, there also exist many polymorphic sites, especially in the intergene spacer region. Although neither *rbcL* data nor *accD*+IGS data alone show informative phylogenetic trees, the combination of these two sequence data results in a more informative tree supported with higher bootstrap value.

In the *accD* gene sequences of Hymenophyllaceae, an interesting phenomenon is observed: stop codon (TAA) occurs in coding region of all of the examined species (the 104th and the 260th positions of amino acids), origi-

Table 2. Morphological characters and reported chromosome numbers

Species	Genera sensu Copeland (1938, 1947)	Morphological characters *1					Reported chromosome numbers	Chromosome basic number
		HT	CW	DL	CD	RE		
Hymenophyllum rolandi-principis Rosenst.	*Rosenstockia*	A	++	+	+	−*2	n=21 (current study)	x=21
H. barbatum (Bosch) Baker	*Hymenophyllum*	A	+	++	++	−	n=21: Japan (Mitui 1966) 2n=42: Japan (Tatuno & Takei 1969)	x=21
H. deplanchei Mett.	*Hemicyatheon*	A	+	−	+	+	not reported	−
H. dimidiatum Mett.	*Meringium*	A	++	++	++	−	n=21: New Caledonia (Braithwaite 1975)	x=21
H. subdimidiatum Rosenst.	*Meringium*	A	++	−	+	−	not reported	−
H. acanthoides (Bosch) Rosenst.	*Meringium*	A	++	++	+	+	not reported	−
H. fuscum (Blume) Bosch	*Amphipterum*	A	+	−	+	+	not reported	−
H. oligosorum Makino	*Mecodium*	A	++	−	++	−	2n=42: Japan (Tatuno & Takei 1969)	x=21
H. wrightii Bosch	*Mecodium*	−	−	−	++	−	n=28: Japan (Mitui 1968*3, 1975, 1986) 2n=c.56: Japan (Mitui 1986) 2n=84: Japan (Tatuno & Takei 1969*4)	x=28
H. polyanthos (Sw.) Sw.	*Mecodium*	−	−	−	++	−	n=27: India (Mehra & Singh 1957) n=28: Japan (Kurita 1976) n=28: Malaya (Manton & Sledge 1954) n=28: Jamaica (Walker 1966) n=28: Vanuatu (Braithwaite 1975) n=28: Trinidad (Walker 1985) n=c.28: Brazil (Löve 1976) 2n=56: Japan (Tatuno & Takei 1969) n=36: India (Irudayaraj & Manickam 1987; Manickam & Irudayaraj 1988)	x=27/28/36
Sphaerocionium pilosissimum (C. Chr.) Copel.	*Sphaerocionium*	B	−	−	+	−	not reported	−*5

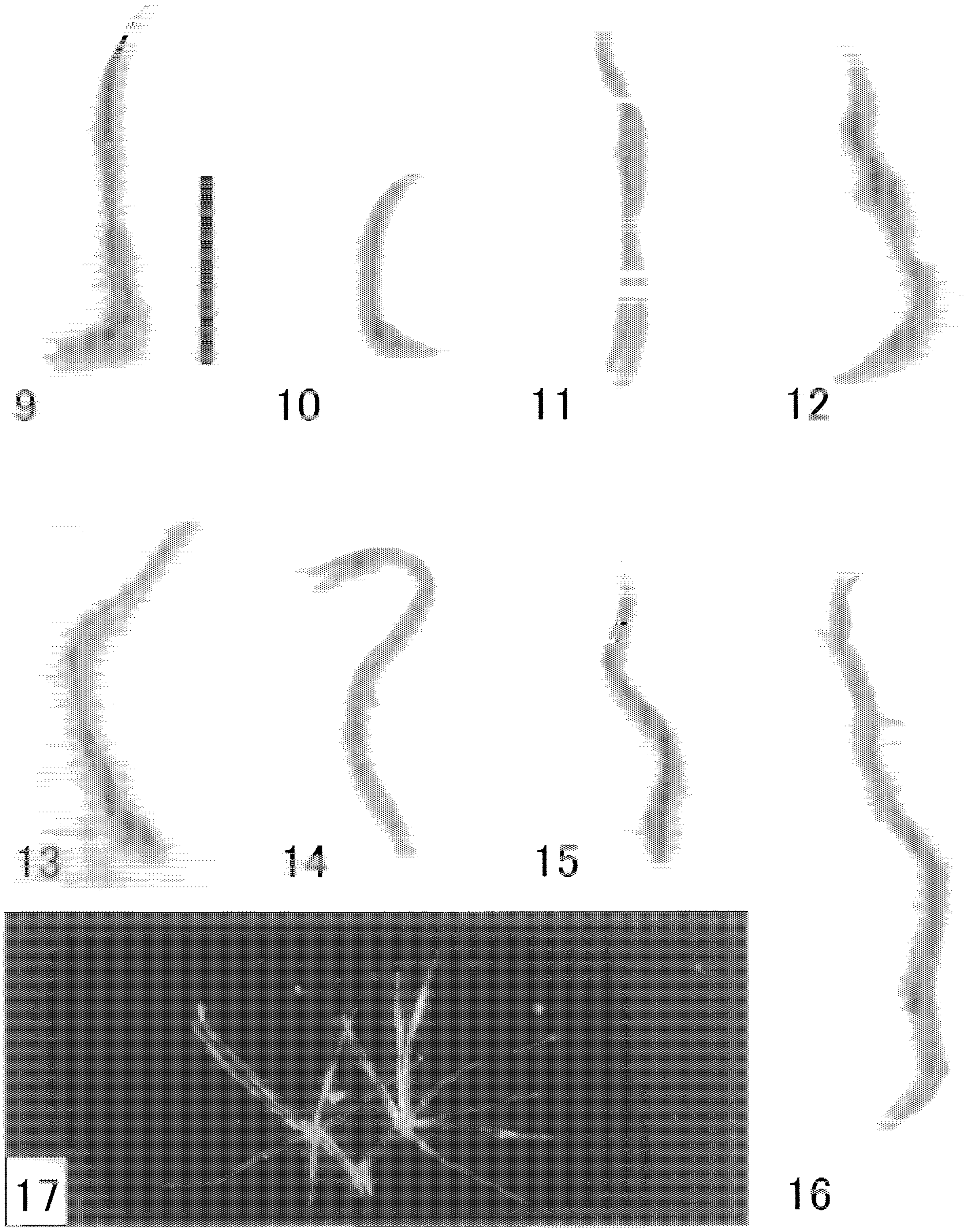
[1] HT: Hair types beneath the frond axis (A: thick, brownish, multicellular hair, B: stellate hair, −: no hair is observed), CW: Thickness of internal cell walls of the frond (++: remarkably thickened, +: rather thickened, −: not thickened), DL: Denticulation of the lips of involucre (++: remarkably denticulated, +: minutely denticulated, −: entire), CD: Cleft depth of involucre (++: cleft nearly to the base, having no obconic basal portion, +: cleft to middle portion, having obconic base), RE: Extruding or included of receptacle (+: receptacle is extruding from lips, −: receptacle is included in lips)

[2] Receptacle of *H. rolandi-principis* is usually included, but is sometimes extruding in old sori.

[3] Counted n=27 at first, but corrected to n=28 in Mitui (1975).

[4] Tatuno & Takei (1969), however, interpreted this number as hypo-octoploid of x=11 from karyotype analysis, Takamiya (1996) considered that it is a triploid of x=28.

[5] Though chromosome number of this species has not been reported, counted basic number of the genus Sphaerocionium is all x=36.

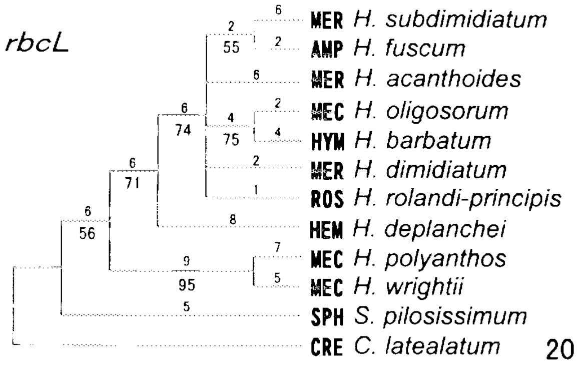


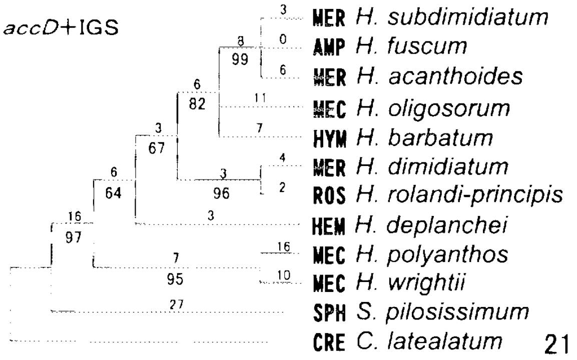
FIGS. 9-17. Hairs underneath rachis in *Hymenophyllum s.l.* 9-16: Brown multicellular hair. 9: *Hymenophyllum rolandi-principis*, 10: *H. dimidiatum*, 11: *H. subdimidiatum*, 12: *H. acanthoides*, 13: *H. barbatum*, 14: *H. oligosorum*, 15: *H. deplanchei*, 16: *H. fuscum*. 17: stellate hair of *Sphaerocionium pilosissimum*. Hairs like these are not observed in the specimens of *H. polyanthos* and *H. wrightii* examined in this study. Bar=0.5mm.

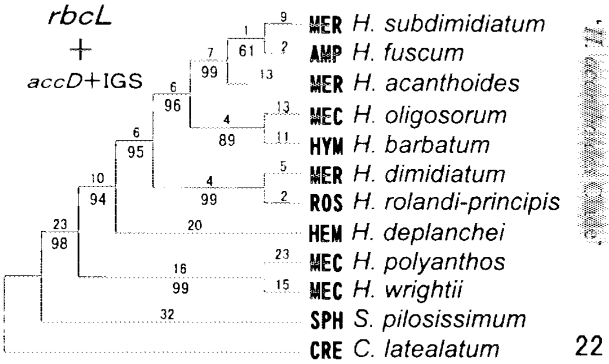
FIGS. 18-19. Gametic chromosomes of *Hymenophyllum rolandi-principis*, n=21. 18. A photograph, 19. A sketch. Bar=10 μm

nally the positions are "CAA", coding Gln, in most of other plants. In land plants including ferns, RNA editing was sometimes observed in the genes on chloroplast and mitochondria DNAs (see Yoshinaga 1997). It is possible to interpret this phenomenon as U to C type RNA editing (Yoshinaga *et al.* 1996), and this type of editing actually occurs in leptosporangiate ferns (Yamada, pers. comm.).

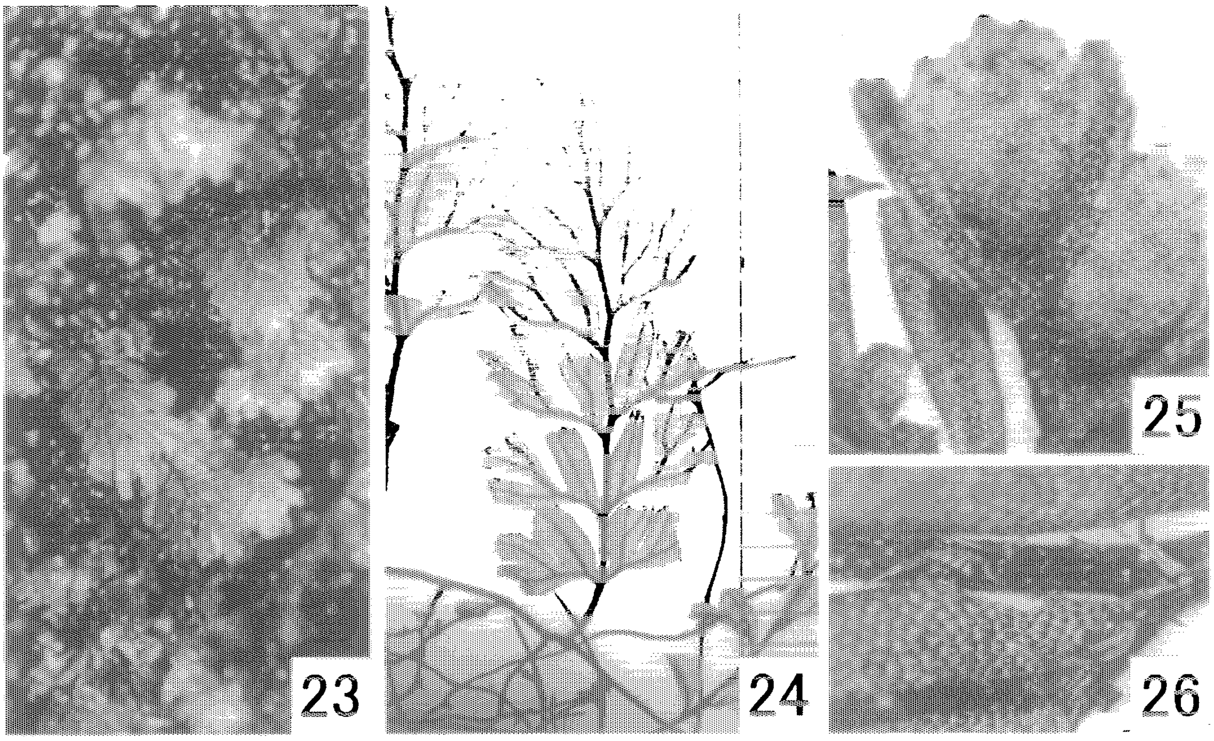
Molecular data suggest that *Hymenophyllum rolandi-principis* should not be an isolated species in *Hymenophyllum s.l.* The most related ally to this in the molecular phylogenetic tree is *H.* (*Meringium*) *dimidiatum* (Figs. 23-26), which grows sympatric to *H. rolandi-principis* on Mt. Panié at about 1400 m above sea level, differing in *rbcL* gene from *H. rolandi-principis* in only one base pair and six positions in *accD* · IGS. Although at first sight these two species are distinct from each other (Figs. 27-28), there are a variety of common features available; thick, brownish, multicellular hairs on the veins underneath, thickend internal cell walls and thickened brown veins. Especially the last is a character that distinguishes these two species from the other members of *Hymenophyllum*. It should be noted, however that the







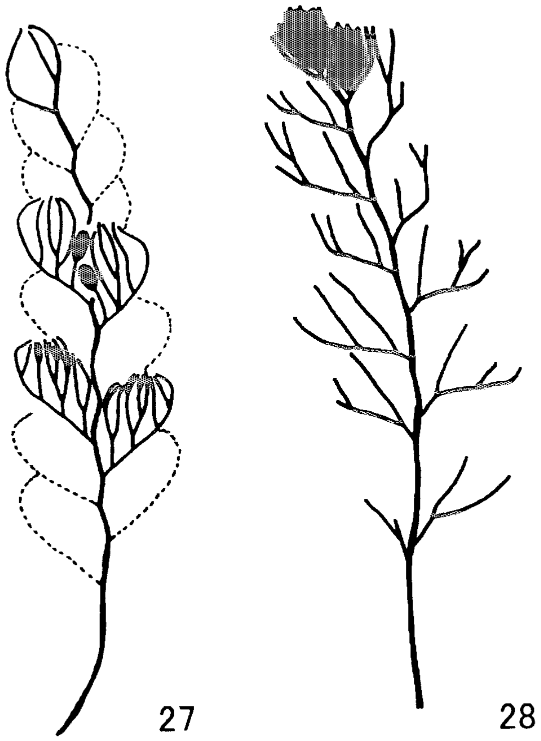
FIGS. 20-22. Strict consensus trees by analysis of maximum parsimony (MP) method with equal weighting. Steps are indicated upon each branches. Bootstrap values are shown under each branch. Genera defined by Copeland (1938, 1947) are shown in bold gothic, MER: *Meringium*, AMP: *Amphipterum*, MEC: *Mecodium*, HYM: *Hymenophyllum s.s.*, ROS: *Rosenstockia*, HEM: *Hemicyatheon*, SPH: *Sphaerocionium*, CRE: *Crepidomanes*. 20. Unique tree from *rbcL* sequences only. 21. Strict consensus tree of two trees from *accD* ·IGS sequences only. 22. Unique tree from the *rbcL* +(*accD*+IGS) sequences.



FIGS. 23-26. *Hymenophyllum dimidiatum* at Mt. Panié. 23. Dwarf epiphytes on a tree trunk, 24. A frond, 25. Sori with remarkably denticulate lips, 26. Dark, thickened veins of frond with brown multicellular hairs.

material of *H. dimidiatum* from Mt. Panié is different from the typical form of this species (cf. Brownlie 1969), in the respect that it is a dwarf form, and looks occupying intermediate position between typical *H. dimidiatum* and *H. pumilio* (cf. Copeland 1938, Plate 45). Consequently it can be argued that *H. dimidiatum* on Mt. Panié is closely related to *H. rolandi-principis*, and that it offers clues to the origin of this particular species.

As to the general classification of the family, the tree based on molecular data shows that a clade containing Copeland's (1938) *Meringium* (*Hymenophyllum acanthoides*, *H. dimidiatum* and *H. subdimidiatum*), *Hymenophyllum s.s.* (*H. barbatum*), *Mecodium* pro parte (*H. oligosorum*) and the other smaller genera such as *Rosenstockia* (*H. rolandi-principis*), *Amphipterum* (*H. fuscum*) and *Hemicyatheon* (*Hymenophyllum deplanchei*); is highly sup-



FIGS. 27-28. Comparison of pinnation patterns in fronds, 27: *Hymenophyllum rolandi-principis*, and 28: *H. dimidiatum*.

ported. Hereafter, this clade is cited as '*H. acanthoides* clade'. Chromosome base number of this group has so far been reported as x=21 (Table 2), and the number of *Hymenophyllum rolandi-principis* corresponds to them. In *Hymenophyllum s.l.*, x=21 has been reported from several species of the following genera sensu Copeland (1938) (cf. Manton & Sledge 1954, Braithwaite 1975); *Meringium, Hymenophyllum s.s., Mecodium* and *Buesia*, the last one is not examined in this study. They are probably involved with '*H. acanthoides* clade'. Dubuisson (1997) stated that cytological data tend to agree with molecular data, in *Trichomanes s.l.*, and this also seems to be true in *Hymenophyllum s.l.* It is fairly reasonable that *Hymenophyllum* (*Hemicyatheon*) *deplanchei* occupies the most basal position of '*H. acanthoides* clade', because *Hemicyatheon* sensu Copeland (1938) is morphologically intermediate between *Meringium* and *Mecodium*. *Sphaerocionium pilosissimum* is placed at the basal position of *Hymenophyllum s.l.* (excluding *Cardiomanes reniforme*), which is supported with high bootstrap value, though the result of Pryer *et al.* (2001) does not show such a strong support from *rbcL* sequences only.

While sampling is not sufficient at this moment, here we try to do some re-evaluation of taxonomical characters of *Hymenophyllum s.l.* In the tree, *Mecodium* sensu Copeland (1938), defined by entire lips of involucres, is polyphyletic. For the examination of characters, we took three Japanese species as examples; *Hymenophyllum wrightii, H. oligosorum* and *H. barbatum*. In Japan, these species have middle- to small-sized fronds, and look alike from a distance. Copeland (1937, 1938) treated *H. oligosorum* as synonym of *H. wrightii*. As for denticulation, *H. barbatum* has remarkably denticulated ultimate segments and lips of involucre, but the others have entire ones. As for hairs, *H. oligosorum* and *H. barbatum* have multicellular hairs on the axes of fronds underneath. The structure of internal cell walls of *H. wrightii* are thin, not pitted, but *H. oligosorum* has thick, pitted cell walls, and those of *H. barbatum* are rather thick, sometimes pitted. Ajima *et al.* (1982) pointed out that *H. oligosorum* and *H. barbatum* resembled each other, except for the existence of denticulation, and that denticulation-like structure was occasionally observed on some segments of *H. oligosorum*.

According to Copeland's system (1938), both *Hymenophyllum wrightii* and *H. oligosorum* belong to *Mecodium* defined by entire segments and involucres, while *H. barbatum* belongs to *Hymenophyllum s.s.* with denticulate segments and involucres. On the other hand, Iwatsuki (1984, 1985) evaluated the structure of internal cell walls and hairs as actual indicators, and clearly distinguished *H. oligosorum* from *H. wrightii*, the former belonging to subgen. *Chilodium* and the latter to subgen. *Craspedophyllum*. The molecular data, which indicate *H. oligosorum* is more closely related to *H. barbatum* than to *H. wrightii*, do not support Copeland's treatment (1938) as far as these species are concerned.

The synapomorphic character shared in '*H. acanthoides* clade' is thick, brownish, multicellular hairs on the axes of fronds underneath especially on rachis. This type of hair is observed in all the species of this clade although hairs of some species are fairly caducous (Figs. 9-17). On the contrary, the species which belong to subgen. *Craspedophyllum* sect. *Integra* have smaller multicellular hairs consisting of a few sub-transparent cells with thin walls (Iwatsuki 1977a, 1985). *Sphaerocionium*, a group placed in the most basal position in the tree, is characterized by stellate hair (Morton 1947, Iwatsuki 1982). The structure of these hairs appears to be a more important character than cell-wall structure which shows gradation. The combination of these two characters will make it possible to define this clade more clearly.

As far as this group is concerned, it is difficult to say that the structure of sori, which is generally treated as an important taxonomical character in the pteridophytes, reflects true phylogenetic lineage. Soral characters such as the denticulation of lips, cleft depth of involucre and extrusion of receptacle, must have evolved in parallel within '*H. acanthoides* clade' (Iwatsuki 1977b). There was an example that the characters traditionally important is not constantly uniform in *Hymenophyllum rolandi-principis*. That is, changes from minutely denticulate involucre lips to remarkably denticulate ones owing to destruction of marginal cells of lips, and from included receptacles to extruding ones by aging.

In spite of its peculiar appearance, *Hymenophyllum rolandi-principis* seems to be safely placed in *Hymenophyllum s.l.*, especially under subgenus *Chilodium* (Iwatsuki 1982, 1984), based on morphological, cytological and molecular data. Therefore, it is not appropriate to recognize *Rosenstockia* as a monotypic genus. Currently available information shows that *H. rolandi-principis* is close to *H. dimidiatum*, at least a local form of this species growing in the same region. It is also suggested that the structure of hairs beneath the frond axes and internal cell walls are more appropriate taxonomical key characters than the structure of sori in defining the species groups of *Hymenophyllum s.l.*

Nomenclature

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Distribution: endemic to Mt. Panié area in northern New Caledonia.

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